

CLAIMS

1. An antimicrobial protein which can be obtained from a fraction of an aqueous extract of *Lyophyllum shimeji* precipitated by the ammonium sulfate precipitation method, wherein said protein has an antimicrobial activity at least against *Rhizoctonia solani* or *Pyricularia oryzae*, and shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method.

2. The antimicrobial protein according to Claim 1, wherein the N-end has the N-end amino acid sequence of SEQ ID NO:3 in the Sequence Listing, Asn Ala Glu Glu Gly Thr Ala Val Pro Tyr Val Pro Gly Tyr His Lys Lys Asn Glu Ile Glu Phe Gln Lys Asp Ile Asp Arg Phe Val.

3. An antimicrobial protein having an amino acid sequence of SEQ ID NO:2 in the Sequence Listing, an amino acid sequence having one to more amino acid mutations therein, or an amino acid sequence having a 50% or more homology with said sequence and showing an antimicrobial activity against *Rhizoctonia solani* or *Pyricularia oryzae*.

4. The antimicrobial protein according to Claim 3 which has an amino acid sequence having a 60% or more homology with the amino acid sequence of SEQ ID NO:2 in the Sequence Listing.

5. The antimicrobial protein according to Claim 3 which has an amino acid sequence having a 70% or more homology with the amino acid sequence of SEQ ID NO:2 in the Sequence Listing.

6. The antimicrobial protein according to Claim 3 which has an amino acid sequence having an 80% or more homology with the amino acid sequence of SEQ ID NO:2 in the Sequence Listing.


7. The antimicrobial protein according to Claim 3 which has an amino acid sequence having a 90% or more homology with the amino acid sequence of SEQ ID NO:2 in the Sequence Listing.

8. The antimicrobial protein according to Claim 3 which has an amino acid sequence having a 95% or more homology with the amino acid sequence of SEQ ID NO:2 in the Sequence Listing.

9. An antimicrobial protein comprising a single polypeptide selected from among a polypeptide having a partial amino acid sequence of 76 to 618 in the amino acid sequence of SEQ ID NO:2 in the Sequence Listing, a polypeptide having an amino acid sequence having one to more amino acid mutations therein, and a polypeptide having an amino acid sequence having a 50% or more homology with said sequence and showing an antimicrobial activity against *Rhizoctonia solani* or *Pyricularia oryzae*, or a combination of these polypeptides.

10. A process for producing an antimicrobial protein according to Claim 1, 3 or 9 comprising:

a step of recovering fraction(s) of an aqueous extract of *Lyophyllum shimeji* precipitated by the ammonium sulfate precipitation method with 75%-saturataion of ammonium sulfate; and

 a step of subjecting said fraction(s) to ion exchange chromatography and recovering fraction(s) eluted at NaCl concentration of 0.05 M to 1 M.

11. A gene encoding an antimicrobial protein according to Claim 1, 3 or 9.

12. The gene according to Claim 11 having a base sequence of SEQ ID NO:1 in the Sequence Listing, a base sequence derived from said sequence by substitution, deletion, insertion and/or addition of one or more bases, or a base sequence capable of hybridizing to said base sequence(s) under stringent conditions.

13. The gene according to Claim 11 having a 50% or more homology with the base sequence of SEQ ID NO:1 in the Sequence Listing.

14. The gene according to Claim 11 having a 60% or more homology with the base sequence of SEQ ID NO:1 in the Sequence Listing.

15. The gene according to Claim 11 having a 70% or more homology with the base sequence of SEQ ID NO:1 in the Sequence Listing.

16. The gene according to Claim 11 having an 80% or more homology with the base sequence of SEQ ID NO:1 in the Sequence Listing.

17. The gene according to Claim 11 having a 90% or more homology with the base sequence of SEQ ID NO:1 in the Sequence Listing.

18. The gene according to Claim 11 having a 95% or more homology with the base sequence of SEQ ID NO:1 in the

Sequence Listing.

19. An oligonucleotide for obtaining a gene encoding an antimicrobial protein originated from *Lyophyllum shimeji* produced by a process comprising:

selecting two domains satisfying the following requirements form the base sequence of the gene encoding the antimicrobial protein of SEQ ID NO:1 in the Sequence Listing:

- 1) each domain consisting of 15 to 30 bases; and
- 2) each domain having 40 to 60% of G+C;

preparing single-stranded DNAs having base sequences which are identical to the base sequences of said domains or complementary thereto, or preparing a single-stranded DNA mixture having degeneracy in the genetic code which ensures that the amino acid residues coded by said single-stranded DNAs are not changed; and optionally modifying the single-stranded DNAs while avoiding damage to the binding specificity to the base sequence of said gene encoding the antimicrobial protein.

20. The oligonucleotide according to claim 19 having a nucleotide sequence of any one of SEQ ID Nos:7 to 12 in the Sequence Listing.

21. A method of isolating the gene according to Claim 11, wherein the method comprises effecting a nucleic acid amplification reaction using a *Lyophyllum shimeji* fruit body cDNA library as a template, and a pair of two oligonucleotides according to Claim 19 as primers to thereby amplify a part of the gene encoding the

antimicrobial protein according to Claim 1, and screening said cDNA library using the amplification product thus obtained as a probe to thereby isolate full-length cDNA clones.

22. A recombinant vector containing the gene according to Claim 11.

23. The recombinant vector according to Claim 22 wherein said vector is an expression vector.

24. A transformant obtained by introducing the recombinant vector according to Claim 22 into a host organism.

25. A process for producing an antimicrobial protein according to Claim 1, 3 or 9 comprising culturing the transformant according to Claim 24 under such conditions as promote the expression of the antimicrobial protein.

26. A recombinant antimicrobial protein obtained by the process according to Claim 25.

27. An antimicrobial agent comprising the antimicrobial protein according to Claim 1 as an active ingredient.